CLAIMS

We claim:

1. Protein conjugates comprising hemoglobin and human serum albumin.

2. Protein conjugates according to claim 1 wherein said conjugates has a molecular weight in a range of 100-300kD.

3. Protein conjugates according to claim 1 wherein said conjugates comprising 1-3 hemoglobin molecules and 1-3 human serum albumin molecules.

4. Protein conjugates according to claim 3 wherein said conjugates comprising 1-2 hemoglobin molecules and 1-2 human serum albumin molecules.

5. Protein conjugates according to claim 4 wherein said conjugates comprising one hemoglobin molecule and one human serum albumin molecule.

6. Protein conjugates according to claim 1 wherein the said hemoglobin is intramolecularly cross-linked.

7. A method for preparing the protein conjugates of claim 1 comprising

preparing stroma-free hemoglobin,

conjugating hemoglobin (Hb) with human serum albumin (HSA), and

purifying said Hb-HSA conjugates.

8. The method of claim 7 wherein the stroma-free hemoglobin is prepared by membrane filtration and ion exchange chromatography, comprising the steps of:

Processing through microfiltration membranes with mean pore size from 0.22 μm to 0.65 μm, followed by ultrafiltration with membranes of molecular weight cut-off from 10kD to 30kD;

The pretreated hemoglobin solution further purified by anion exchange chromatography in flow-through mode at 4-10℃, with 10-50mM buffer, pH 6.6- 8.5, using 0.25-10% polyethylene glycol (PEG) 400-4000 as an escort.

9. The method of claim 7 wherein Hb and HSA conjugation methods is either one-step or two-step coupling, wherein in two step coupling, the cross-linker reacts first with one protein either in solution or on solid medium, then reacts with another protein in solution.

10. The method of claim 7 wherein the purification of Hb-HSA conjugates comprising either one, two or three of the methods selected from ion exchange chromatography, ultrafiltration and gel filtration chromatography.

11. A method for using the hemoglobin conjugates of claim 1 wherein said conjugates are used as blood substitutes.